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Isolation and Trail-Following Bioassay of a Decay Fungus Associated with *Reticulitermes hesperus* Banks (Isoptera: Rhinotermitidae)

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Abstract.—The brown-rot decay fungus *Oligoporus balsameus* (Pick) (Basidiomycetes: Polyporaceae) was isolated from a Douglas-fir timber inhabited by the western subterranean termite *Reticulitermes hesperus* Banks. Decay tests with white fir and red alder blocks were performed on malt agar media. Decayed white fir blocks were extracted by sequential soaking in petroleum ether, chloroform, and methanol, and these extracts assayed individually and in combination for their ability to induce trail-following in *R. hesperus* workers. The chloroform fraction, and combinations containing that fraction, elicited significant trail-following. However, the level of activity was much less than that reported for extracts of termite body parts containing trail pheromone, indicating either quantitative or qualitative differences in the active compound(s).

Introduction

Termite behavior and survival on cellulosic materials can be affected by the presence of decay fungi. For example, Hendee (1935) found that *Zootermopsis angusticollis* (Hagen) fed on decayed Monterey pine, *Pinus radiata* D. Don, and Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, consumed more wood and sustained less mortality than those fed on sound wood. Smythe et al. (1971) also reported greater feeding by *Reticulitermes flavipes* (Kollar) on decayed than on sound wood, but found that effects on termite survival varied greatly depending upon the species of wood, species of fungus, extent of decay, and whether or not the mycelium had been killed by oven drying. *Reticulitermes* spp. have also been observed feeding on basidiocarps in rotten logs (Waller et al., 1987), indicating a direct dietary role.

Effects on termite survival may be due to the nutritive value of the fungus itself, increased availability of nitrogen and soluble carbohydrates in the decayed wood, or neutralization by the fungus of toxic substances in the wood (Becker, 1971; La Fage and Nutting, 1977; Smythe et al., 1971). In tests with both sound wood and wood decayed by the brown-rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr., Carter et al. (1972) and Carter and Smythe (1973) noted that differences in diet were reflected by differences in the composition of free amino acids and fatty acids in *R. flavipes*.

Whatever the nutritional significance of fungal decay, effects on termite behavior are well documented and have been reviewed by Amburgey (1979), Becker (1976), and Sands (1969). Esenther et al. (1961) first reported that an aqueous extract of

Pinus monticola Dougl. wood decayed by *G. trabeum* attracted or arrested *R. flavipes, Reticulitermes virginicus* Banks, and *Nasutitermes columbicus* (Holmgren). Several attractive compounds are present in *G. trabeum* extracts (Smythe et al., 1967b; Ritter and Coenen-Saraber, 1969; Watanabe and Casida, 1963). One of these compounds, (Z,Z,E) 3, 6, 8-dodecatrien-1-ol, is considered identical to a trail pheromone isolated from *R. virginicus* (Matsumura et al., 1968, 1969).

Although many termite species are attracted or arrested by extracts of *G. trabeum, Reticulitermes hesperus* Banks is affected to a lesser extent than other *Reticulitermes* species (Allen et al., 1964; Matsumura et al., 1972; Smythe et al., 1967a). *R. hesperus* is the most common subterranean termite along the west coast of North America, and a serious structural pest. Possible behavioral effects on *R. hesperus* by fungi co-occurring with this termite have not previously been investigated.

The chemical ecology of *R. hesperus* is not only of heuristic value. Naturally occurring attractants and repellents offer possible alternatives to the current reliance upon soil treatments using toxic chemicals for termite control. The development of toxic baits attractive to subterranean termites is one promising example (Esenther and Beal, 1979). Successful application of such techniques to the control of *R. hesperus* is dependent upon the isolation and identification of appropriate behavioral chemicals. Reported here is the isolation of the decay fungus *Oligoporus balsameus* (Peck) (= *Polyporus balsameus*) (Basidiomycetes: Polyporaceae) (Gilbertson and Ryvarden, 1985) from wood inhabited by *R. hesperus,* and the laboratory trail-following bioassay of solvent extracts of wood decayed by this fungus.

Materials and Methods

Source of Fungus and Insects.—The decay fungus was collected and cultured in August 1982, from a Douglas-fir *(P. menziesii)* 2×8 inch form board along a residential driveway in Berkeley (Alameda County), California. This board was embedded in the soil and inhabited by western subterranean termites, *R. hesperus.* These termites were used in on-going studies in our laboratory, necessitating the collection of a second colony for the behavioral assays with fungal extracts.

The second colony of *R. hesperus* was collected from Douglas-fir floor joists in a residence in Oakland (Alameda County), California, in July 1983. These were maintained in a humidity chamber (Grace, 1986) until behavioral assays were performed in May 1984. Because of their predominance in foraging activities, only workers (externally undifferentiated individuals older than the third instar, as determined by size) were used in these assays.

Fungal isolations were performed from visibly sound internal wood ca. 2 mm below the surface of *R. hesperus* galleries. Thin slivers of wood (ca. $5 \times 5 \times 2$ mm) were surface sterilized by a 30 second emersion in 0.5% sodium hypochlorite. These were placed on 2% malt agar in petri dishes. Seven days after inoculation, white mycelial growth (1–2 cm diameter) had radiated outward from some of the slivers. The leading edge of the mycelium was transferred to a clean plate (2% malt agar). Hyphal tips were retransferred four more times at 7–14 day intervals before transfer to slant tubes, which were refrigerated for 11 months before the decay tests. Under microscopic examination, clamp connections characteristic of basidiomycete fungi were clearly visible on the hyphae.

Decay Tests.—The ability of the fungus to decay wood was determined in agar-block decay tests, in which blocks of wood were placed on agar containing active fungal mycelia.

Glass bottles containing 75 ml of 2% malt agar were inoculated with 25 mm^2 slices of agar from fungal culture plates. Sterile strips (ca. 30 × 35 mm) of blotting paper (feeder strips) were placed on the surface of the agar as a nutrient source. Ten days after the fungal inoculation, sterile 26 × 26 × 10 mm blocks of white fir (*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.) (Pinaceae) sapwood (n = 21, av. wt. = 2.29 ± 0.04 g) were placed flat on the feeder strips with the end grain fully exposed to the fungal mycelium. White fir was the standard gymnosperm used in these tests. 18 blocks were exposed to the fungus and 3 blocks were placed on sterile agar as controls. Blocks were oven-dried before and after exposure to measure the weight loss due to fungal decay.

Single treatment blocks were removed at 2, 4, 6, and 12 weeks and the weight loss was measured. At 12 weeks, an additional treatment block and a control block were also removed, oven-dried and weighed to confirm the degree of decay.

Ten white fir treatment blocks were removed at 12 weeks, air-dried for 6 weeks on a wire rack, and weighed after desiccation for 24 hours. Five of these air-dried blocks were then extracted with solvents in order to determine whether behaviorally active compounds were present. The remaining white fir treatment blocks were removed at 15 and 17 weeks to extend the decay test to four months.

A second agar-block decay test was performed simultaneously with red alder (*Alnus rubra* Bong.) (Betulaceae) sapwood blocks to test the ability of the fungus to decay a representative hardwood (angiosperm). Six blocks were inoculated as previously described and removed and weighed at 4, 7, 10, and 12 weeks.

Solvent Extraction and Behavioral Assays.—Five white fir blocks, decayed to ca. 19% weight loss (av. wt. = 1.77 ± 0.04 g) by a 12 week exposure in the agar-block decay test and air-dried, were extracted by sequential soaking in petroleum ether, chloroform, and methanol for 48 hours each at room temperature (22–24°C). These solvent extracts were assayed for their ability to induce trail-following in *R. hesperus* as described by Grace et al. (1988). Assays were performed on a glass surface, uniformly illuminated by overhead fluorescent lighting (13.5–19.5 foot-candles), at room temperature (22–24°C).

In each assay, a straight 200 mm artificial trail (1–2 mm in width) was drawn on Monroe No. 41 tracing paper with a microliter syringe containing 4 μl (microliters) of solution. The solvent was allowed to evaporate for ca. 15 seconds, and a single *R. hesperus* worker was deposited from a glass vial onto one end of the trail. As a single measure of recruitment to the trail and orientation upon it, the distance traveled on the trail in a 30 second interval was recorded. Each insect and each trail were used only once to preclude trail reinforcement or behavioral conditioning.

The three solvent fractions were assayed both individually and in combination with each other to test for synergistic responses. Control trails drawn with a 1:1:1 mixture of the three solvents were also assayed. Distances traveled by 25 workers in each treatment were analyzed by the analysis of variance (ANOVA), and means compared by the Ryan-Einot-Gabriel-Welsch Multiple F Test, $\alpha = 0.05$ (SAS Institute, 1982).

Results and Discussion

The field-collected basidiomycete rapidly decayed both the gymnosperm and angiosperm (Table 1), the decayed wood had a brown cubical appearance, and the culture tested oxidase negative by no color change when drops of gum guaiac solution (0.5 g per 30 ml ethanol) were applied, all suggesting that it was a brown-rot

Table 1. Percent weight loss in ca. 2 g white fir and red alder blocks after inoculation with *Oligoporus balsameus* isolated from Douglas-fir inhabited by *Reticulitermes hesperus*.[a]

Weeks After Inoculation	Percent Weight Loss ± SEM	
	White Fir	Red Alder
2	0%	
4	8	7%
6	15	
7		20
8	11	
10		28
12	22 ± 3[b] (n = 2)	29 ± 4 (n = 3)
15	27	
17	34	

[a]Agar-block decay test. Blocks oven-dried before and after exposure to fungus. Weight of one block (n = 1) unless otherwise noted.

[b]Mean weight loss of 10 additional blocks air-dried for six weeks was 17 ± 2%.

fungus, rather than a white-rot type or a secondary saprophyte. It was subsequently identified as *O. balsameus,* a decay fungus which has not previously been associated with subterranean termites. In fact, only a few natural associations between termites and brown-rot fungi have been reported (Esenther et al., 1961; Williams, 1965). Waller et al. (1977) recently reported the isolation of thirty basidiomycetes from logs infested by *Coptotermes formosanus* Shiraki and *Reticulitermes* spp., all of which caused white-rot decays.

Hendee (1934) isolated six undetermined basidiomycetes from colonies of *R. hesperus* and two other termite species in California. However, her study focused on the role of fungi in the diet of *Zootermopsis angusticollis* (Hagen) (Hendee, 1935), and she did not attempt to distinguish between decay fungi and secondary saprophytes. Thus, to our knowledge, this represents both the first isolation of *O. balsameus* from wood adjacent to subterranean termite galleries and the first definitive isolation of a brown-rot fungus associated with *R. hesperus.*

When solvent extracts of the white fir blocks, decayed to ca. 19% weight loss, were assayed for their ability to elicit trail-following in *R. hesperus,* only trails containing the chloroform fraction were significantly different from the solvent controls (Table 2). Workers traveled the greatest distance (18.12 ± 0.83 mm) on trails drawn with the chloroform extract alone. The responses to the combination of the chloroform and petroleum ether fractions, and to the combination of all three fractions, did not differ significantly from the response to the chloroform fraction alone. These two combinations also did not differ significantly from the combination of chloroform and methanol. Neither the petroleum ether nor the methanol fraction alone, nor the combination of these two fractions, elicited any significant response. Thus, there was no evidence of activity in the petroleum ether and methanol fractions, nor of synergism among the fractions.

Table 2. Mean distance traveled in 30 seconds by *Reticulitermes hesperus* workers on artificial trails drawn with solvent fractions of *Oligoporus balsameus* decayed white fir blocks.

Solvent Fraction(s) on Trail[1]				
Solvent Control	Petroleum Ether	$CHCl_3$	CH_3OH	Mean Distance ± SEM (mm)[2,3]
	X			3.28 ± 0.28 c
		X		18.12 ± 0.83 a
			X	5.76 ± 0.42 bc
	X	X	X	14.92 ± 0.47 ab
	X	X		10.24 ± 0.53 abc
		X	X	7.12 ± 0.40 bc
	X		X	2.48 ± 0.20 c
X				1.84 ± 0.17 c

[1]Blocks were extracted by sequential soaking in Pet. ether, $CHCl_3$, and CH_3OH.

[2]Mean of 25 assays with individual *R. hesperus* workers.

[3]Means followed by different letters are significantly different (ANOV, REGW Multiple F Test, $\alpha = 0.05$).

Although statistically significant, the trail-following response elicited by the chloroform fraction was still substantially less than that reported by Grace et al. (1988) with extracts of *R. hesperus* sternal glands. On the basis of the weight of material extracted, the activity of the decayed white fir would be ca. 1/120,000 that of extracted insect sternites.

This low level of trail-following activity may have either a qualitative or quantitative basis. Extraction of decayed sawdust, rather than intact 2 g blocks, may yield more of the active material. Fungal culturing on Douglas-fir rather than the white fir test substrate employed as a standard in this study, may also enhance activity. Production of fungal compounds is known to vary with the wood substrate (Esenther and Beal, 1979) and with the specific fungal isolate (Amburgey and Smythe, 1977).

Most studies of associations between fungi and subterranean termites (Rhinotermitidae) have investigated the effects of known fungi from laboratory cultures or previously identified fungal metabolites, rather than using fungi isolated from field-collected wood to examine natural associations. However, it was field observations of *R. flavipes* foraging behavior and the subsequent isolation of eight fungi (five basidiomycetes) by Esenther et al. (1961) that led to the identification of the potent trail-following compound (Z,Z,E) 3, 6, 8-dodecatrien-1-ol. From both a basic and applied standpoint, examinations of natural fungal associations with termite species may prove more fruitful than screening known compounds or laboratory fungal cultures.

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